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## THE EPIDERMAL GLANDS OF *LYGODACTYLUS* (GEKKONIDAE, LACERTILIA)


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Recent histological and ultrastructural studies have shown that the squamate epidermis is quite different from that of other vertebrates. In snakes and lizards, unique "epidermal generations" (Maderson, 1967) can be identified. These units, whose periodic appearance, maturation, and loss are reflected in a series of differing histological pictures at various times in the sloughing cycle, are made up of six (and sometimes more) different cell populations arising sequentially from an apparently homogeneous stratum germinativum. Some of these populations, e.g. those that form the superficial *Oberhautchen* component of the $\beta$-layer, or the innermost "clear layer," normally comprise only a single contiguous cell layer over the entire body surface. Others, e.g. the cells comprising the remainder of the $\beta$-layer, or the $\alpha$-layer, are represented by numerous cell layers, although there may often be considerable variation from one genus or species to another or even from one part of the body or from one scale to another.

Although the details of the morphology of the squamate epidermis have only recently been reported (Maderson, 1965a, b, 1966b, 1967; Maderson and Licht, 1967; Roth and Jones, 1967), certain specialized modifications of this organ system have been described. Conclusive demonstration of an associated nerve supply (Miller and Kasahara, 1967) has confirmed the identity of so-called "sense organs" in lizards, described by earlier workers (Schmidt, 1920; Preiss, 1922). These studies, and those of the climbing organs of gekkonid and anoline lizards (Maderson, 1964a; 1966a; Ruibal and Ernst, 1965; Ernst and Ruibal, 1966; Lillywhite and Maderson, 1968) have shown that although the primary function of the *Oberhautchen* is to facilitate sloughing


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(Maderson, 1966b), this cell layer has undergone considerable adaptive specialization in various lizard groups.

Specialization of the squamate epidermis is not restricted to the formation of sensory and climbing organs; gland-like structures have also been reported (Cole, 1966a, b). While I was assembling material for a study on the so-called "glandular escutcheon scales" (Taylor and Leonard, 1956) of the Central American gekkonid *Gonatodes*, Dr. E. E. Williams of Harvard University drew my attention to the recorded presence of apparently similar structures in the East African gekkonid *Lygodactylus* (Pasteur, 1964). Preliminary examination of a few specimens revealed fundamental differences in the microscopic anatomy of the organs in the two genera. The structure of the escutcheon scales of *Gonatodes* proved to be not only of intrinsic interest but also contributed considerably to our understanding of the pattern of development of a squamate epidermal generation (Maderson, 1967). From a comparative cytological point of view, the superficially similar structures in *Lygodactylus* are more of a curiosity than of fundamental significance, but a detailed account of their structure is warranted for other reasons. First, it provides another example of the remarkable evolutionary potential of the squamate epidermis for the formation of specialized structures; second, it represents a further contribution to our knowledge of gekkonid anatomy; and finally, it helps to shed some light on the problem of the homology of the various types of "pores" found in gekkonids (Kluge, 1967).

## MATERIAL AND METHODS

Through the courtesy of Dr. E. E. Williams of Harvard University and Dr. J. Peters of the Smithsonian Institution, I was able to obtain 29 male specimens of *Lygodactylus* spp. Loveridge's (1947) and Pasteur's (1964) statements to the effect that only males show the specialized glandular scales and pre-anal pores were confirmed by dissection and histological examination of 12 females from the same sources. The species were identified as *L. gutturalis, L. picturatus, L. p. keniensis* and *L. fischeri* from various known localities in East Africa. No significant differences in the structure of the glands were seen between species.

Pieces of skin were removed from the posterior abdominal surface just anterior to (but sometimes including) the pre-anal pores, or from the ventral aspect of the femoral region, which shows similarly modified scales (see below). Histological examination revealed that there are similarly modified scales between the line of

pre-anal pores and the vent, but there are no external indications of their presence. The history of some of the material was uncertain but it was probably fixed in the field in formalin and later stored in 70 per cent alcohol. The specimens were dehydrated in alcohol, cleared in chloroform, embedded in 56°C paraffin, and cut at 7$\mu$ vertical to the skin surface, either transverse or longitudinal to the scale axis (see below). Sections were mounted serially, a few from each ribbon being mounted unstained and examined by phase contrast. The remainder were stained in Ehrlich's hematoxylin and eosin, or aniline blue-orange G, or Masson's trichrome (Gurr, 1958). The material in the pre-anal pores proved to be exceptionally difficult to cut, giving confirmatory evidence of its nature (see below).

## OBSERVATIONS

### The Scales

*Macroscopic appearance.* The posterior abdominal, ventral femoral, and ventral tibio-fibular scalation is quite different in the males (Pl. 1, fig. 1, left) than in the females (Pl. 1, fig. 1, right). In the male, 2 to 3mm anterior to the vent, there is a transverse row of slightly enlarged scales on whose outer surface (Maderson, 1964b) is seen a circular opening about 0.3 mm across, in which a plug of material may be seen. There are 7 to 9 of these so-called "pre-anal" pores depending on the species (Pasteur, 1964); not infrequently there may be one or more which is considerably larger than the others. In the male, the darker pigmentation of these regions is distinctive. The scales lying in a blunt V-shaped area leading forwards from the line of pre-anal pores are the same shape (regularly trapezoid, the short axis lying parallel to the body axis, with little overlapping of successive elements) as the more anterior abdominal scales, but are distinctly darker. On the femoral region there are patches of scales of essentially similar shape (Loveridge, 1947), which are very much darker than those on the abdomen. This femoral patch grades into non-specialized, lighter-colored scales anteriorly (Pl. 1, fig. 1, left) but ends sharply posteriorly. There is a similarly modified patch on the ventral aspect of the tibio-fibular surface (Loveridge, 1947). The individual scales all show a more lightly pigmented marginal area; this is seen in histological sections to indicate the lateral margins of the specialized zones on each outer scale surface (Maderson, 1964b).

*Microscopic appearance.* As has been described elsewhere for *Gonatodes* (Maderson, 1967), the glandular scales of *Lygodactylus*

show a number of different histological conditions which can be interpreted as manifesting changes in association with periodic sloughing. This assumption will be followed in the descriptions below of four significantly different conditions which are important in interpreting the morphological structure of the specialized scales.

All the specimens examined showed conditions which could be interpreted as belonging to the "proliferation-renewal phase" (Maderson, 1965a, b, 1966b, 1967; Maderson and Licht, 1967). That a large museum sample should show 100 per cent of the material in the proliferative phase is very unusual in the author's experience. Mr. Allen Greer of Harvard University collected the 21 specimens examined of the Kenyan *L. picturatus* during an ecological study (Greer, 1967), and he provided me with further data regarding this material. The animals were caught by hand, or shot with dust-shot, and then fixed in formalin within at least four hours. Experience in collecting Australian gekkonids (Maderson, unpublished data) suggests that the stress of capture initiates a new epidermal cycle (Maderson, 1967), with the result that material obtained from animals kept in captivity for three or four days will always show various stages in epidermal proliferation. Although Mr. Greer tells me that he rarely saw *L. picturatus* actually sloughing, on the few occasions that he did so, they were engaged in removing material from their body surfaces with their teeth. This behavior pattern has been described in a number of squamate species (Bustard and Maderson, 1965). In *Gekko gecko* (Chiu, Phillips and Maderson, 1967) and *Anolis carolinensis* (Maderson and Licht, 1967) the complete physical removal from the body of the material to be shed is accomplished in a very short time (something less than 5 hours and often during the night [Maderson, unpublished data]). Although the material examined here did not permit an estimate of the time occupied by the various stages of epidermal differentiation (Maderson and Licht, 1967), the picture which emerges is that *Lygodactylus* either sheds very frequently or else has a cycle in which the "resting phase" (Maderson, 1967) is extremely short or absent. It would be of interest to know to what extent this is associated with the probable ecological and behavioral significance of the glands and pores (Cole, 1966a, b).

In median longitudinal section the individual scales are seen as triangular structures arising from the body surface with very little overlapping of adjacent elements (Pl. 5, fig. 2). In transverse section the picture varies depending on the level along the scale axis, but in general one sees a rather wide-based structure, again with little overlap of adjacent scales. The outer scale surface (Maderson, 1964b) is always distinctly concave with the deepest portion

towards the posterior distal region of the scale; it is in this concavity that the specialized epidermal material is seen. In gross and low-power microscopic appearance there is a very general similarity to the escutcheon scales of *Gonatodes* (Maderson, 1967).

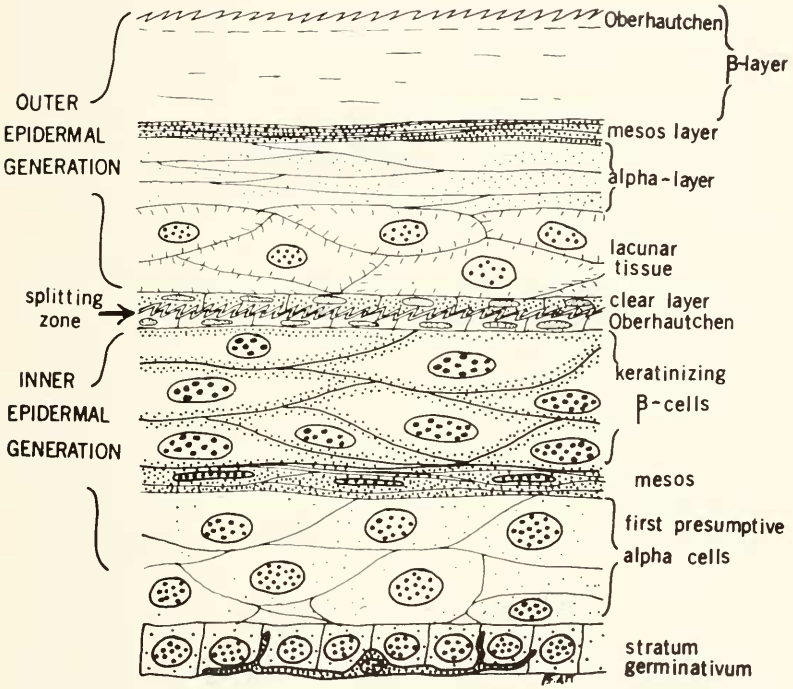


Figure 1. Schematic representation of a generalized squamate epidermis several days before sloughing is due to take place. (Figure taken from Maderson [1967] and reproduced here by kind permission of the editors of Copeia.)

To avoid undue repetition of references in the text of the following descriptions, all the terms used are those discussed and defined in previous works (Maderson, 1965a, 1966b, 1967; Maderson and Licht, 1967). This nomenclature is shown in schematic form in Figure 1.

Condition One (6 specimens); Pl. 1, figs. 2-4.

Plate 1, figure 2 shows a sagittal section through a scale taken from the anterior margin of the specialized abdominal region where the gland development is smallest; an enlarged portion of the low-power view is shown in Plate 2, figure 4.

The surface of the body is formed from a homogeneous, chromophobic tissue which shows no indications of nuclear remains or cell outlines; this is the $\beta$-layer of the outer epidermal generation. The extreme superficial surface of the $\beta$-layer is formed by the characteristic *Oberhautchen* which in gekkonids (Maderson, 1966b, 1967; Ruibal and Ernst, 1965) is always seen as a series of minute vertical spinules (Pl. 1, fig. 4). Towards the distal portion of the outer scale surface, the *Oberhautchen* is separated from the normal $\beta$-material described above by a quite different material (Pl. 1, fig. 4). The latter is basophilic in hematoxylin stained sections and stains a patchy orange and blue in sections stained with aniline blue/orange G. This suggests that the material is of a keratinaceous nature, as adjudged by its affinity for orange G (Gurr, 1958), but with a mixed complement of possible mucoprotein material which stains with the aniline blue. There are also copious deposits of melanin granules. This is here termed the $\beta$-gland material, for reasons which will be discussed later. Initial examination posed the problem of how this could be regarded as a "gland" if the material was confined between the *Oberhautchen* (which is a very strong unit in spite of its being composed of only a single cell layer [Maderson, 1966a]) and the rest of the $\beta$-layer. Although examination of serial sections showed that often only the edges of the material, proximally, distally, and laterally were covered by the *Oberhautchen*, it was assumed that histological preparation had destroyed a contiguous central portion. The proof that this is not so will be presented later, and in fact there is an approximately circular area on the outer scale surface where the gland material is directly exposed to the external environment; this is due to a regional non-development of the *Oberhautchen*. This is the first time that the author has ever seen a situation where there is not a continuous *Oberhautchen* over the whole epidermis so as to cover the entire body surface. Even on the inner scale surface and in the hinge region where the $\beta$-layer is often difficult to recognize histologically, ultrastructural studies (Ernst and Ruibal, 1966) have shown that the $\beta$-layer is represented here by the *Oberhautchen* alone.

The $\beta$-layer invariably separates from the underlying tissues during histological preparation, and the artifactual gap is traversed by refractive strands which represent the only histological indications of the mature mesos layer. This is often difficult to identify in geckos but is marked by an X in Plate 1, figure 4.

Beneath the mesos layer is a region of lamellar chromophilic material containing dense deposits of melanin, showing no indications

of nuclei except for the occasional pycnotic figure seen as a presumptive α-cell becomes incorporated into the tissue (Maderson and Licht, 1967) at its very base: this is the α-layer of previous accounts. No further changes occur in the histological appearance of the tissues described thus far and they will not be mentioned again in subsequent descriptions.

Beneath the α-layer are two or more rows of flattened or rectangular cells. The outermost layer(s) (*plto*, Pl. 1, fig. 4) are not continuous over the entire outer surface of the scale where new gland material is seen (see below) but are easily recognizable in other regions (see below). This represents the presumptive lacunar tissue which plays an important role in the maturation of an epidermal generation prior to shedding (Maderson, 1967; Maderson and Licht, 1967). The innermost layer (*pclo*, Pl. 1, fig. 4) is not readily recognizable as a typical gekkonid clear layer (Maderson, 1966b, 1967) at this time, and only its later development really reveals its identity (see below). The tissues and layers thus far described comprise a "complete" outer epidermal generation (Maderson, 1967).

Between the presumptive clear layer and the stratum germinativum is a cell mass comprising tight-packed polygonal cells. In sagittal section (Pl. 1, fig. 4) there is a slight indication of oblique orientation of the cells towards the scale apex, but in transverse section (Pl. 1, fig. 3) their orientation is vertical away from the stratum germinativum. The nuclei are rounded in the early stages of development (Pl. 1, fig. 4) but tend to become ovoid later (Pl. 1, fig. 3). The cytoplasm presents a slightly filamentous appearance but is often floccular in the early stages of development. There is only a single recognizable cell type in the region at this time. This mass of cells represents the first indications of the new gland material which will eventually be exposed to the external environment after the next slough. As the number of cells in this region increases, the basally situated epidermal melanocytes extend dendrites far above the stratum germinativum and deposit melanin granules into the cells (Pl. 1, fig. 3).

The stratum germinativum consists of cuboidal cells when the cell mass above it first appears (Pl. 1, fig. 4), but there is a gradual change to a more columnar condition later (Pl. 1, fig. 3).

Condition Two (8 specimens); Pl. 2, figs. 1-2.

A longitudinal section of a major glandular scale (Pl. 2, fig. 1) shows that the most significant change from the previous condition is that the new gland material is now seen to be an integral cell mass confined distally by a region of highly flattened

cells and proximally by the stratum germinativum. The flattened cells (Pl. 2, fig. 1) beneath the darkly chromophilic α-layer will eventually form a 2 to 3 layered lacunar tissue, which will be bounded basally by a clear layer, and the flattened layer beneath this (the one forming the outermost boundary of the mass of new gland material cells) will become the *Oberhautchen*. Two points must be emphasized here. First, the cell layers which are here termed the "presumptive clear layer" (*pclo*, Pl. 2, fig. 2) and the "presumptive *Oberhautchen*" (*pObi*, Pl. 2, fig. 2) are not yet recognizable on the basis of their cytology, only by their topographical position. Second, Plate 2, figure 2 shows a section through a lateral part of a scale where a distinct lacunar tissue and clear layer/*Oberhautchen* complex forms (see below); the picture in a median sagittal plane would be very different. Here the base of the mature α-layer would lie directly against the uppermost cells of the mass of new gland material. The high power micrograph of the lateral margin as seen in transverse section (shown as Pl. 2, fig. 2) shows some of the cytological detail visible at this time. In this particular field the lacunar tissue is not developed (it is of variable occurrence and distribution in lacertilians [Maderson, 1966b; Maderson and Licht, 1967]). The *Oberhautchen* (which is not as regular in shape here as over the general body surface of other gekkonids [Maderson, 1966b]) is just recognizable under oil immersion by the slightly thickened outermost cell membranes. The overlying clear layer is also distorted in most regions of the glandular scales in *Lygodactylus*, as compared with that of other gekkonids (Maderson, 1966b).

The extraordinary variations in cell shape, distribution of elements, etc., described above would make identification of the lacunar tissue, clear layer, and *Oberhautchen* very tenuous, were it not for the fact that in serial sections the various regions and layers can be traced along the adjacent "semi-specialized" (see later) and unspecialized scales. In these last locations, the histology approaches the conditions described for the normal body epidermis of *Gekko gecko* during the early proliferative phase (Stages 2 and 3 [Maderson, 1966b]). Certain features of these adjacent regions, which provide clues as to the homologies of the various cell types seen in the specialized scales of *Lygodactylus*, will be discussed later.

Condition Three (13 specimens); Pl. 2, figs. 3, 4; Pl. 3, figs. 1, 2.

In these specimens one sees the laying down and subsequent maturation, i.e. keratinization, of cells which can be recognized as

a $\beta$-, a mesos, and a partially formed $\alpha$-layer of the inner epidermal generation (Maderson, 1967). The pattern of histological changes occurring in the lacunar tissue and clear layer of the outer epidermal generation supports the general assumption that the gland scales resemble a much-modified form of the typical gekkonid body epidermis during the pre-sloughing period (Stages 3-5 [Maderson, 1966b]). Apart from the gland material, all of the regions which are identified and discussed below are contiguous with similar regions of the epidermal surface of adjacent unmodified scales which resemble exactly the general body epidermis described elsewhere (Maderson, 1966b).

A low-power view of a transverse section through a glandular scale when the first presumptive $\beta$-cells become visible is shown in Plate 2, figure 3, and the region indicated by the vertical strip is shown in Plate 3, figure 1. The latter shows a portion of the scale where the new gland material is not bounded by an *Oberhautchen* and there is no clear layer or lacunar tissue associated with the outer epidermal generation, so that the outermost layer of new gland cells abuts directly onto the base of the $\alpha$-layer, which is shown artifactually split away in Plate 2, figure 3.

The mass of cells termed the "new gland material" (Pl. 2, fig. 3) shows two distinct types of cell. The most predominant form is a polygonal shaped cell with a slightly thickened membrane and relatively large central ovoid or rounded nucleus lying in a densely filamentous cytoplasm (Pl. 3, fig. 1). These filaments are slightly basophilic in hematoxylin stained sections and also show a strong affinity for orange G. The only similar cytological picture which the author has ever seen is in chick scales during the later embryonic stages when the characteristic avian $\beta$-keratin is being laid down in that location (Baden and Maderson, unpublished data). For this reason and to distinguish the glands of *Lygodactylus* from the escutcheon scales of *Gonatodes* (the formative stages of which are *totally* different [Maderson, 1967]), I suggest that these abdominal glands in *Lygodactylus* should be termed "$\beta$-glands." In a series of specimens I have observed the gradual death of these cells, as indicated by the increasing frequency of pycnotic figures (Pl. 3, fig. 2; Pl. 4, fig. 3). The filaments become less and less distinct and the entire cell eventually becomes either finely basophilic (hematoxylin) or totally orange in orange G-stained material. The conspicuous thickening of the cell membranes which normally (Maderson, 1966b, 1967) characterizes the cytogenesis of the subjacent presumptive $\beta$-cells is not seen here; this may be due to

the fact that the gland material never becomes quite as homogeneous in appearance in its mature state as does the actual $\beta$-layer of the outer epidermal generation (see p. 6).

The second cell type seen in the mass of presumptive gland material has a quite different cytology. In hematoxylin and eosin sections the cytoplasm is floccular and is faintly eosinophilic. The central nucleus is about the same size and shape as that of the other cells. In sections stained with aniline blue the floccular appearance takes on the form of a dense blue mesh-work with occasional deep-staining blue granules. These cells are scattered randomly between the filament-filled cells in the main body of the new gland material (Pl. 3, fig. 1; *X*) but tend to be concentrated in clumps around the lateral, proximal, and distal margins of the material (Pl. 3, fig. 3; Pl. 4, fig. 2; *X*). These positions correspond to portions of the mature material where the blue-staining (with aniline blue/orange G stain) is most dense. On adjacent scales (Pl. 4, fig. 1), cells of a similar nature are seen, but their distribution and relative abundance are different. Here a distinct *Oberhautchen* with characteristic spinules is seen; beneath this are 1 to 3 layers of the blue-staining granular cells (Pl. 4, fig. 1; *gc*), and beneath these, in turn, normal presumptive $\beta$-cells in various stages of maturation are seen. In other instances (further away from the main "gland area" on the abdominal and limb surfaces) occasional cells of this type are seen. In the latter, the cytoplasm resembles that of the "protruding" *Oberhautchen* cells seen in the epidermis of *Gekko gecko* (Maderson, 1966b, p. 43), except that whereas the latter definitely showed spinules on the outer membrane, in *Lygodactylus* the cells contact the clear layer cells directly with no indication of spinule development. A final point which will be of significance in homologizing the various cell types is the fact that in *Lygodactylus* (and numerous other gekkonid species [Maderson, unpublished data]) *definite Oberhautchen* cells, i.e. cells which eventually form the outermost surface of the body, which develop in association with overlying clear layer cells, and which show various degrees of spinule development, can be seen to contain granules which stain with aniline blue; these granules are not seen in the underlying presumptive $\beta$-cells.

Material showing this condition confirms the regional absence of the *Oberhautchen* covering over much of the gland material; the very sharp discontinuity of this covering is seen in transverse (Pl. 3, fig. 2) and sagittal (Pl. 3, fig. 3) sections (see arrows). The clear layer and lacunar tissue are also absent from those regions where there is no *Oberhautchen*. A slight problem is presented by

the morphology of the cell layer indicated by *Y* in Plate 3, figure 3. This layer lies between the main mass of new gland cells and the easily recognizable presumptive β-cells which are nearly fully keratinized. Plate 3, figure 3 shows that this cell layer is very different from the underlying cells and does not contain the conspicuous filaments seen in the gland cells. In many ways it resembles a typical gekkonid *Oberhautchen* except for a complete absence of any sign of spinule development. However, the total lack of any indication of topographic continuity between this layer and the *Oberhautchen* (of characteristic gekkonid form) finishing at the arrow (Pl. 3, fig. 3) suggests that the cytological similarity is misleading. I suggest that there is a large area where there is no development of a characteristic *Oberhautchen;* this point is important in assessing the homologies of the various cellular elements in the glandular scales.

The morphological appearance and subsequent maturation of the cell layers termed the lacunar tissue and the clear layer are exactly the same as those described in *Gekko gecko* (Maderson, 1966b, 1967). Subsequent development of the elements comprising the rest of the inner epidermal generation (presumptive β-cells, presumptive mesos layer, presumptive α-cells) is also quite typical, and details of their structure will be found elsewhere (Maderson, 1966b, 1967; Maderson and Licht, 1967). The columnar appearance and relative chromophobia of the stratum germinativum cells as the mesos layer is laid down (Pl. 4, figs. 1, 2, 4) (Maderson and Licht, 1967) are emphasized.

Condition Four (2 specimens); Pl. 2, fig. 5; Pl. 4, fig. 4; Pl. 5, fig. 1.

One specimen showed a very late phase in the keratinization of the new β-layer of the inner epidermal generation, with subjacent presumptive mesos cells showing pycnotic figures, and beneath these numerous presumptive α-cells (Maderson, 1966b, 1967; Maderson and Licht, 1967). Another (Pl. 5, fig. 1) showed a definite "pre-sloughing" condition with the inner epidermal generation having a mature β-layer, a mature mesos layer showing as refractive strands, as described earlier, and with the first indications of a new α-layer beneath. Beneath the 3 to 4 layers of presumptive α-cells which would normally be incorporated into the α-layer during the post-sloughing period (Maderson, 1966b, 1967; Maderson and Licht, 1967), there were some cells which resembled exactly the first indications of the next mass of new gland cells, as described earlier. In the absence of more material one hesitates

to make definitive statements, but it is probable that this genus never shows a typical "resting phase" in the sloughing cycle.

Sloughing, i.e. the physical removal of the outer epidermal generation from the body surface, involves the separation of the clear layer of the outer generation from the *Oberhautchen* of the inner generation (cf. Fig. 1; Maderson, 1966b, 1967; Maderson and Licht, 1967) as seen in Plate 5, figure 1, left. Where the clear layer/*Oberhautchen* complex is absent (over the central portion of the new gland material), the separation occurs at the base of the old $\alpha$-layer, which lies directly on top of the new gland material (Pl. 5, fig. 1, right). Cell shapes are still visible in the latter at this time, and the material appears to occupy a greater vertical depth than does the mature material; this may be due to drying of the material once it is exposed to the external environment. The staining characteristics of the gland material in the pre-slough specimen are exactly those of the exposed material (see p. 6).

## The Pre-anal Pores

*Macroscopic appearance.* The external appearance of the pore openings has already been described above (p. 3). Dissection of the integument in the pre-cloacal region reveals the inner portions of the pores as apparently simple sacs running directly anteriad from the opening for a distance of 1.2 to 1.4 mm. The sacs lie between the subcutaneous tissue and the ventral musculature. They are surrounded by fat.

*Microscopic appearance* (Pl. 5, figs. 2-4; Pl. 6, figs. 1-4). A median longitudinal section through the opening of a pre-anal pore is shown in low power view in Plate 5, figure 2. The scales anterior and posterior to the scale on which the pore opening is situated show $\beta$-glands in "condition three" as described above. It is noteworthy that the rest of the epidermis on either side of the pore lumen is not modified in any way (Pl. 5, figs. 3 and 4) and its structure is that which has been described elsewhere (Maderson, 1966b) as a stage four condition of the normal gekkonid body epidermis. Plate 5, figure 4 gives a clear picture of the slightly modified condition normally seen towards the inner scale surface and hinge region where there is quite extensive development of a lacunar tissue (*lto*) in which the characteristic basophilic granules (keratohyalin? [see discussion, Maderson, 1966b]) are to be seen. Following the epidermis from the scale surface into the mouth of the pre-anal pore, one sees the striking discontinuity of all the component layers and regions seen on the normal epidermis; these

are marked by arrows in Plate 5, figures 3 and 4. The most rigorous examination of the pre-anal pore walls (see below) reveals no homologous layers or any resemblance to them, except for a stratum germinativum. The major portion of the mouth of the pre-anal pore in Plate 5, figure 2 is empty, but numerous fragments of the original "plug" of material can be seen. This material is very much harder to cut than either the $\beta$-gland material, the escutcheon gland material of *Gonatodes* (Maderson, 1967a), or iguanid gland material (Maderson, unpublished data). It is very slightly eosinophilic and stains fairly densely with orange G. The shape of the individual fragments suggests that they are single, hardened cells, but there are no signs of nuclear remains in the mature material. The histological structure of the lining and of the lumen contents varies as one follows down the length of the gland.

At about the region where the gland turns rather sharply forward (Pl. 5, fig. 2), the lumen is normally filled with mature material as described above and the lining shows the histological picture seen in Plate 6, figure 1. There is a cuboidal stratum germinativum and above it 2 to 3 layers of loosely arranged, slightly flattened cells. There is no indication that new cells, contributing to the actual pore material, arise from this region.

About half-way down the length of the pore, immature glandular material may be seen (Pl. 6, fig. 2). This stains deeply with orange G, individual cells can be recognized, and some contain pycnotic nuclei. There is no obvious separation between these cells and the mature material described above; they merge imperceptibly into one another.

In the anterior portion of the gland the histological picture is quite different. Just beyond the region described above there are masses of tightly packed cells of irregular polygonal shape with a strongly heterogeneous fibrillar cytoplasm (Pl. 6, fig. 3). The nuclei are all viable and the cytoplasm appears to contain tightly packed fibrillar elements lying in an effectively floccular matrix. The fibrils stain intensely with orange G, suggesting a keratinaceous nature, while the background is patchy blue, indicating an uptake of aniline blue. There is no indication of thickening of the cell membranes. The figure suggests that intercellular spaces are common; this is an artifact of sectioning, since certain regions show this appearance, but in other parts the cells are closely packed.

At the base of the pore are seen cells which can reasonably be interpreted as "mother cells." Their very specific appearance gives a clear indication of how the cells described above are formed. Lines of columnar cells reach into the pore lumen from the stratum

germinativum. Those nearest the germinal layer (Pl. 6, fig. 4) show a distinct division into three parts. Nearest the germinal cells, the relatively large, ovoid nucleus can be seen. Next, there is a region of cytoplasm with a slightly amorphous appearance, sometimes suggesting irregular droplets or granules; this region stains conspicuously with orange G. The distal portion of the cell contains a large droplet (Pl. 6, fig. 4; arrow); this is slightly eosinophilic but stains particularly intensely with aniline blue. As the cells move out into the lumen, and begin to move towards the pore mouth, the orientation of the three portions is lost, and the distinct droplet soon disappears. One assumes that the patchy blue staining visible in cells further up the lumen results from a disintegration of the large droplets and a spreading of the material throughout the cell cytoplasm.

All the various stages described above were visible at various levels wherever a portion of pre-anal gland material had been sampled with the overlying scales; there was no indication of any cycle of activity.

## DISCUSSION

There have been comparatively few studies of specialized "glandular structures" in lizards (Cole, 1966b), and those works which are available are for the most part concerned with follicular glands, such as femoral pores (Cole, 1966a).

Taylor and Leonard (1956) considered the "escutcheon scales" of *Gonatodes*, and I have recently re-examined the problem in the light of our present knowledge of the squamate epidermis (Maderson, 1967). The specialized scales of *Gonatodes* and of *Lygodactylus* are similar in that their pattern of formation is intimately associated with the periodic sloughing cycle of the epidermis, but here the similarity ends.

The major differences between the mature and developing gland material in the two genera can best be expressed in the following table:

| *Gonatodes* (Maderson, 1967) | *Lygodactylus* |
|---|---|
| 1. Specialized scales restricted to a V-shape anterior to cloaca. (N. B. In other sphaerodactyline forms [*Sphaerodactylus*, Thomas and Schwartz, 1966] specialized scales may be found on the femoral region also.) | 1. Specialized scales seen on posterior abdomen, immediately anterior to the cloaca, and on the ventral aspects of the hind limbs. |

| | |
|---|---|
| 2. Gland material is borne on the surface of the β-layer of the outer epidemal generation and has an incomplete *Oberhautchen* running *beneath* it. | 2. Gland material is found *in* the β-layer of outer epidermal generation with an incomplete *Oberhautchen* partially covering it. |
| 3. Gland material is derived from the *basal* portion of the epidermal generation which was lost at the previous slough. Thus in the "resting phase" there are tissues in the epidermis derived from two *different* epidermal generations. | 3. Gland material is derived from the *superficial* portions of the epidermal generation upon which it is borne. As there is some doubt that a true "resting phase" ever exists, there may always be tissues from two different generations present in the epidermis, but for quite different reasons (see text, p. 11). |
| 4. Single cell type seen in developing gland material. | 4. Two distinct cell types seen in developing gland material. |
| 5. Staining of keratinaceous elements of the gland material suggests an affinity with the α-layer | 5. Staining of keratinaceous elements of gland material suggests an affinity with the β-layer. |
| 6. At sloughing, splitting zone over glandular material is at base of the ∝-layer of the outer epidermal generation, leaving a portion of the outer epidermal generation behind. | 6. At sloughing, entire outer epidermal generation is lost. Although the splitting zone does occur at the base of the α-layer of the outer generation over the gland material, this is due to a partial non-development of the lacunar tissue and clear layer of the outer generation. |

These differences are so fundamental that I suggest the specialized scales in the two genera are not in any way homologous.

The problem of the relationship of the specialized scales to the pre-anal pores is slightly complicated by the possibility that this particular genus has no true resting phase in its sloughing cycle. Cole (1966a) suggests that there is a continuation of the normal epidermis (referred to in his paper, pp. 125-126, as the "stratum corneum") into the femoral gland mouth in *Crotaphytus*, which splits the "plug" into an outer portion which is lost at shedding and an inner portion which takes the place of the latter. This would imply that glandular activity is directly correlated with the epidermal sloughing cycle. Cole (1966a) also suggests that there is some evidence that there is annual cyclical activity of the iguanid femoral

pores. In a study of *Gonatodes* (Maderson, 1967), I have indicated that gekkonids typically shed very frequently throughout the year. *If* there is indeed *annual* cyclical activity of the gland (? in association with the reproductive cycle), then one would have expected that at some stage in the evolution of true pores or glands, their pattern of cyclical protein synthesis would have had to become *independent* of the rest of the epidermis. In *Gonatodes* (Maderson, 1967) there are morphological indications of how this might have occurred. As far as one can tell in *Lygodactylus* there is no indication of a separation between the completely mature pre-anal pore material and the cells still undergoing differentiation, so that the pre-anal pores in this genus resemble a tube of tooth-paste from which material is slowly squeezed out. It is unprofitable to speculate on this point in the absence of material collected throughout the year in an attempt to demonstrate a true annual cycle of activity in the pre-anal pores.

Apart from the problem of the relative activity of the pre-anal pores and the $\beta$-glands, there is the problem of the relationship between the two types of structure. Here I would unhesitatingly suggest that the $\beta$-glands represent an ancestral condition of the pre-anal pores *in this genus* (see below). The striking discontinuity of the *Oberhautchen* has been emphasized. If, in association with gland development on the scales, one can see the absence of a superficial layer, there is no logical reason to deny the possibility that subjacent layers could also be reduced and eventually lost during evolution. In fact I have demonstrated that this process has already begun in the absence of the lacunar tissue and clear layer from the central portion of the $\beta$-glands. Thus, reference to Plate 5, figure 2 shows that one only has to imagine that the rest of the epidermal generation beneath the developing gland material (presumptive-$\beta$-cells, presumptive mesos cells and presumptive $\alpha$-cells) could disappear and one would be left with a "shallow pre-anal pore." Furthermore, the figure shows that if a particular portion of the individual scale, i.e. the portion over which the gland material forms, invaginated deeper and deeper into the subcutaneous tissues, a pre-anal pore type of structure would be developed. The epidermis of the scale upon which the pore opens is notably different from the adjacent scales showing no indication of normal gland development; this would be homologous with the extreme unmodified margins of the $\beta$-gland scales which show a quite typical "normal body epidermis" structure (Maderson, 1966b). A consideration of probable intergeneric homologies of scales and pores

depends on the interpretation of the various component cell types which will be discussed next.

The presence of two distinct cell types in the β-glands has been emphasized. Although the chemistry of epidermal proteins is a highly complex field, and consideration thereof is not pertinent to an article of this kind, it is permissible to suggest that those cells which show a definite affinity for orange G are synthesizing a keratin, while those showing an affinity for aniline blue are synthesizing a mucoprotein of some description. The vertebrate epidermis has the capacity to form either keratins or mucins, as evidenced by studies on the structure of the amphibian epidermis (Parakkal and Matoltsy, 1964), and under certain experimental conditions (New, 1963). It seems that in *Lygodactylus* β-glands the two functions are carried on within different cells. However, there is evidence from adjacent scales in this and other genera which suggests that *Oberhautchen* cells (or at least, cells belonging to the same portion of the epidermal generation as the *Oberhautchen* or presumptive β-cells) are capable of both activities. Whether these modified cells occurring either singly or in a double or triple epithelium (Pl. 4, fig. 1) should be regarded as "*Oberhautchen*" cells is a semantic problem; one would prefer to retain the term only for those cells which show the characteristic development of spinules or setae on the outermost membrane (Ruibal and Ernst, 1965; Ernst and Ruibal, 1966). Within the pre-anal pores, there is no indication of two distinct cell types. There is however evidence of localization of mucoprotein synthesis and keratin synthesis within a single cell. This then should be regarded as a specific specialization of the tendency seen in *Oberhautchen* cells on the non-specialized scales of this and other genera. In the femoral pores of *Gekko gecko* (Maderson, unpublished data) such "mixed-function" cells are not seen; here there are cells which only stain positively for keratin and cells which only stain positively for mucoprotein. A "non-association" with normal epidermal proliferation is also seen in *Gekko gecko,* as indicated by the discontinuity of all the normal layers and regions of the inner epidermal generation at the femoral pore mouth, exactly as has been described here in *Lygodactylus*. Although there is a need for detailed studies of the basic anatomy and possible cyclical activity in glands from a variety of gekkonid types, it is suggested that the problem of direct homology between the pre-anal pores of *Lygodactylus* and the femoral pores of *Gekko gecko* will be one of semantics. Granted that there are situations where "single-function" and "mixed-function" cells may be identified, the fundamental relationship of the

pore cells to the normal epidermal generation is more important. Thus I suggest that there is some evidence that lygodactyline β-glands are definitely homologous with the pre-anal pores of that genus and with the femoral pores of *Gekko gecko* — all materials deriving from the outermost portions of the epidermal generation — but there is no evidence of homology with the type of glandular scale seen in the sphaerodactyline gekkonids, where the glandular material derives from the innermost portions of an epidermal generation. The absence of pre-anal pores from sphaerodactylines (Kluge, 1967) makes it impossible to prove or disprove Kluge's (1967) statement that the escutcheon scales in these forms are "almost certainly modified pre-anal organs" (p. 18). Taylor and Leonard (1956) suggested that the escutcheon scales of sphaerodactylines represented structures from which pre-anal organs in other groups were derived; the analysis of the cellular components in *Gonatodes* (Maderson, 1967) and the evidence presented here suggest that this is unlikely. In the present state of knowledge one can only surmise that the specialized scales thus far described in sphaerodactylines appear to be the result of an independent evolutionary pathway from those found in *Lygodactylus* and *Gekko gecko*. Further comment on the phylogenetic implications of scale, pore, and gland structure must await detailed analysis of material from a variety of forms.

## SUMMARY

1. The histological structure of the scalation of the abdominal, femoral and tibio-fibular surfaces and pre-anal pores of 29 male specimens of *Lygodactylus* spp. has been studied.

2. The epidermis of the scales in these regions shows a number of histological conditions which can be interpreted as representing different stages in the histogenesis of the integument in association with periodic sloughing. All the characteristic elements of a typical squamate "epidermal generation" are represented. There is present an additional region consisting of a keratinaceous material with a mucoprotein component; this material develops in association with the β-layer of the epidermal generation and lies between the *Oberhautchen* and the subjacent β-layer. In the mature state the material is partially exposed to the external environment by a unique regional non-development of the *Oberhautchen*.

3. The pre-anal pores are simple invaginations of single scales running forwards in the ventral body wall musculature. There is no obvious indication of any cyclical activity, and it would appear that

the activity of these glands is quite independent of the sloughing cycle of the rest of the body covering.

4. Comparison of the structure and development of the modified scales with what is known of superficially similar "escutcheon scales" in sphaerodactyline gekkonids suggests that the two organ systems are of quite independent evolutionary origin, there being no evidence of any homology between them. To prevent further confusion in this context, it is suggested that the organs described here in *Lygodactylus* should henceforth be termed "$\beta$-glands."

5. Comparison of the structure of the $\beta$-glands with the pre-anal pores suggests that the former present a definite possible ancestral form of the latter *in this genus.*

6. The problem of the evolutionary relationships of gekkonid glands and pores is briefly discussed. The small amount of available evidence suggests the possibility that whereas there is a broad basis for assuming an homology between the pre-anal pores in *Lygodactylus* and *Gekko gecko,* no homology of any description can be assumed with the escutcheon scales of sphaerodactylines. The basis for assessing homologies of these epidermal structures in gekkonid lizards should depend on a consideration of the fundamental relationship of the "gland" material to the epidermal generation.

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## ABBREVIATIONS USED IN PLATES

| | |
|---|---|
| *βi* | β-layer of inner epidermal generation |
| *βo* | β-layer of outer epidermal generation |
| *αo* | α-layer of outer epidermal generation |
| *clo* | Clear layer of outer epidermal generation |
| *clo/Obi* | Clear layer/*Oberhautchen* complex |
| *f* | Filaments |
| *gc* | Granular cells |
| *GM* | Exposed glandular material |
| *H* | Hinge region |
| *ISS* | Inner scale surface |
| *lto* | Lacunar tissue of outer epidermal generation |
| *m* | Melanin granules |
| *mi* | Mesos layer of inner epidermal generation |
| *mo* | Mesos layer of outer epidermal generation |
| *NGM* | New glandular material |
| *Obi* | *Oberhautchen* of inner epidermal generation |
| *Obo* | *Oberhautchen* of outer epidermal generation |
| *OSS* | Outer scale surface |
| *pβi* | Presumptive β-layer of inner epidermal generation |
| *pαi* | Presumptive α-layer of inner epidermal generation |
| *pclo* | Presumptive clear layer of outer epidermal generation |
| *plto* | Presumptive lacunar tissue of outer epidermal generation |
| *pmi* | Presumptive mesos layer of inner epidermal generation |
| *pObi* | Presumptive *Oberhautchen* of inner epidermal generation |
| *sg* | Stratum germinativum |

# PLATES

## PLATE 1

Figure 1. Posterior abdominal and sub-caudal surfaces of *Lygodactylus picturatus* male (left) and female (right). Note the darker scales on the abdominal, femoral and tibio-fibular surfaces of the male as compared with the female. The small square of tissue missing from the abdominal surface of the male is seen in figs. 2-4. Scale divisions in mm.

Figure 2. Low-power view of sagittal section through two successive scales from the region shown in fig. 1. The portion outlined is shown in fig. 4. This specimen shows the simplest form of "Condition One" (text pages 5-7). Hematoxylin and eosin stain.

Figure 3. High-power view of transverse section through a more typical "$\beta$-gland" from the abdominal surface showing a later "Condition One" than in figs. 2 and 4. The $\beta$-layer and the exposed gland material are absent. Hematoxylin and eosin stain.

Figure 4. Oil immersion montage of region outlined in fig. 2.

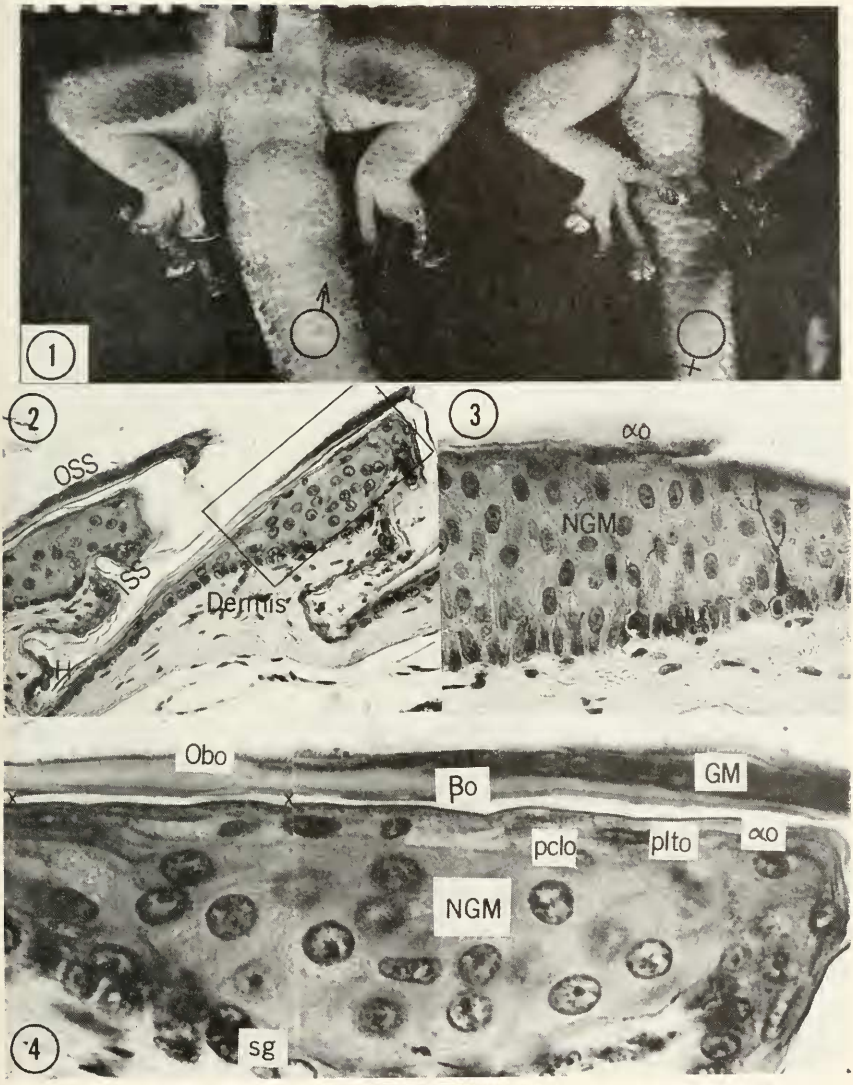



PLATE 1

## PLATE 2

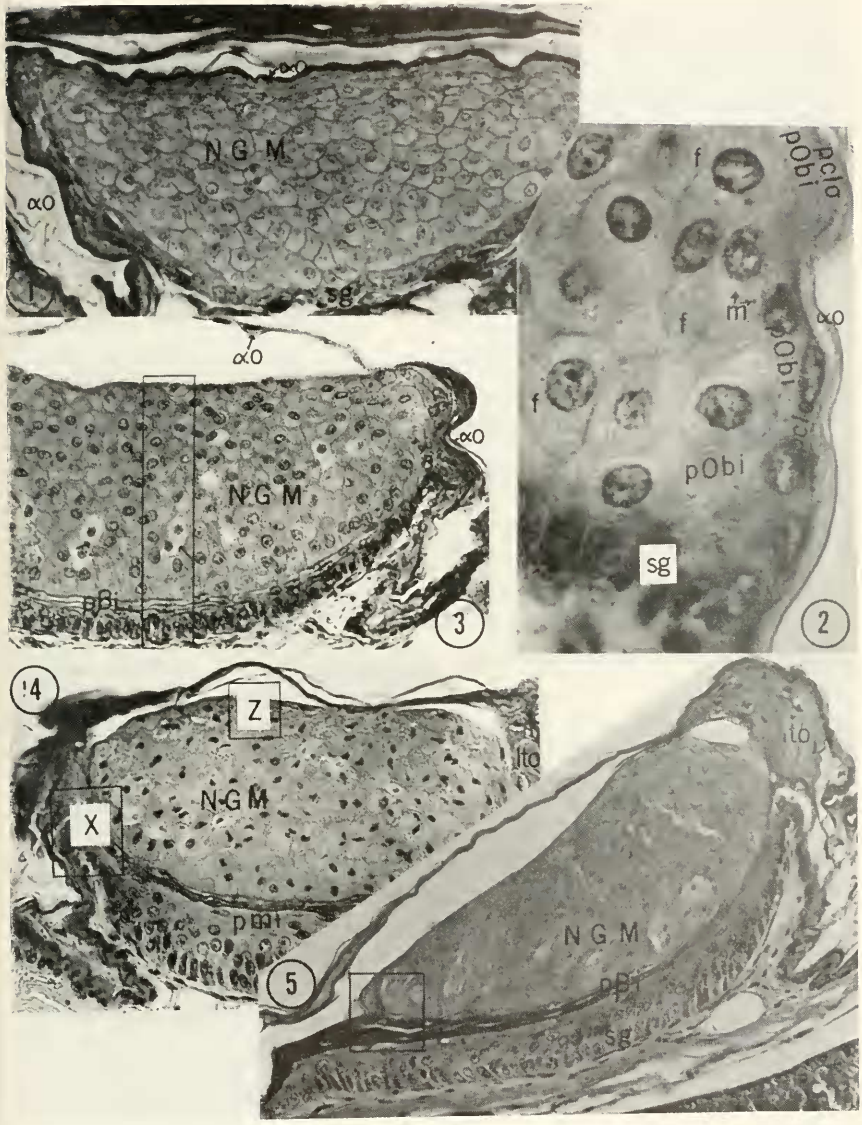
Figure 1. Sagittal section through epidermis of a specialized scale from the femoral region showing "Condition Two." Masson's trichrome stain.

Figure 2. Extreme lateral margin of a specialized scale from the femoral region showing "Condition Two" as seen in transverse section. This shows the layers of cells which will later be recognizable as the lacunar tissue, the clear layer and the *Oberhautchen*. The $\beta$-layer of the outer epidermal generation (represented only by the *Oberhautchen* in this region [Ernst and Ruibal, 1966]) is not shown. Hematoxylin and eosin stain.

Figure 3. Transverse section through a specialized scale from the abdominal surface showing an early "Condition Three." The $\beta$-layer of the outer epidermal generation and the exposed gland material are not shown. The region enclosed by the rectangle is shown in Pl. 3, fig. 1. Hematoxylin and eosin stain.

Figure 4. Transverse section through a specialized scale from the abdominal surface showing a mid-late "Condition Three." The $\beta$-layer of the outer epidermal generation is not shown. The region enclosed by rectangle marked *X* is shown in Pl. 3, fig. 2, and that marked *Z* is shown in Pl. 4, fig. 3. Aniline blue-orange G stain.

Figure 5. Median sagittal section through a specialized scale from the abdominal surface showing a very late "Condition Three" or early "Condition Four." The $\beta$-layer of the outer epidermal generation is not shown. A region comparable to that enclosed by the rectangle is show in Pl. 3, fig. 3. Aniline blue-orange G stain.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5

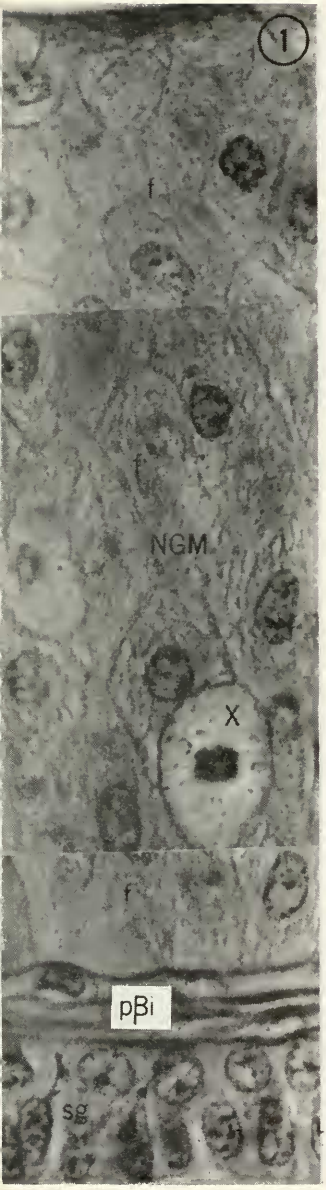
PLATE 2

## PLATE 3

Figure 1. Oil montage of region indicated by rectangle in Pl. 2, fig. 3. Note the conspicuous filaments (*f*) in the more numerous cell type in the new gland material (*NGM*) and the occasional cell with granules (*X*). The outermost portion of the new gland material abuts directly onto the base of the α-layer of the outer epidermal generation (not shown).

Figure 2. Region comparable to that indicated by rectangle marked *X* in Pl. 2, fig. 4. Note the pycnotic nuclei visible in the new gland material and the sharp discontinuity of the *Oberhautchen* of the inner epidermal generation marked by an arrow.

Figure 3. Region comparable to that indicated by a rectangle in Pl. 2, fig. 5. The clear layer of the outer epidermal generation (*clo*) is recognizable by pycnotic nuclei. The sharp discontinuity of the new *Oberhautchen* is indicated by an arrow. Compare with Pl. 4, fig. 2.


1
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f
NGM
X
f
pβi
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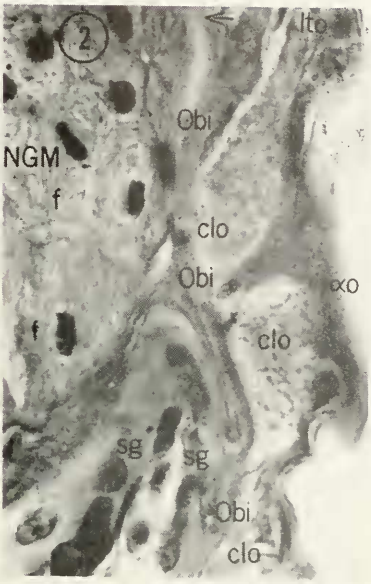
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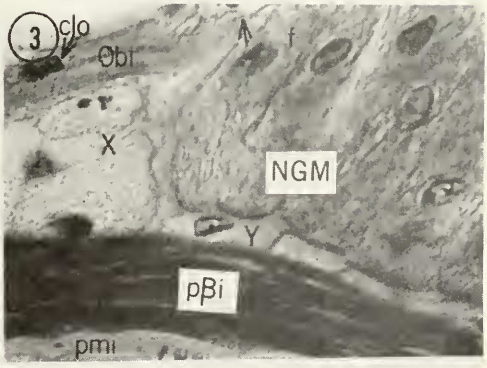
3
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Obi
f
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Y
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PLATE 3

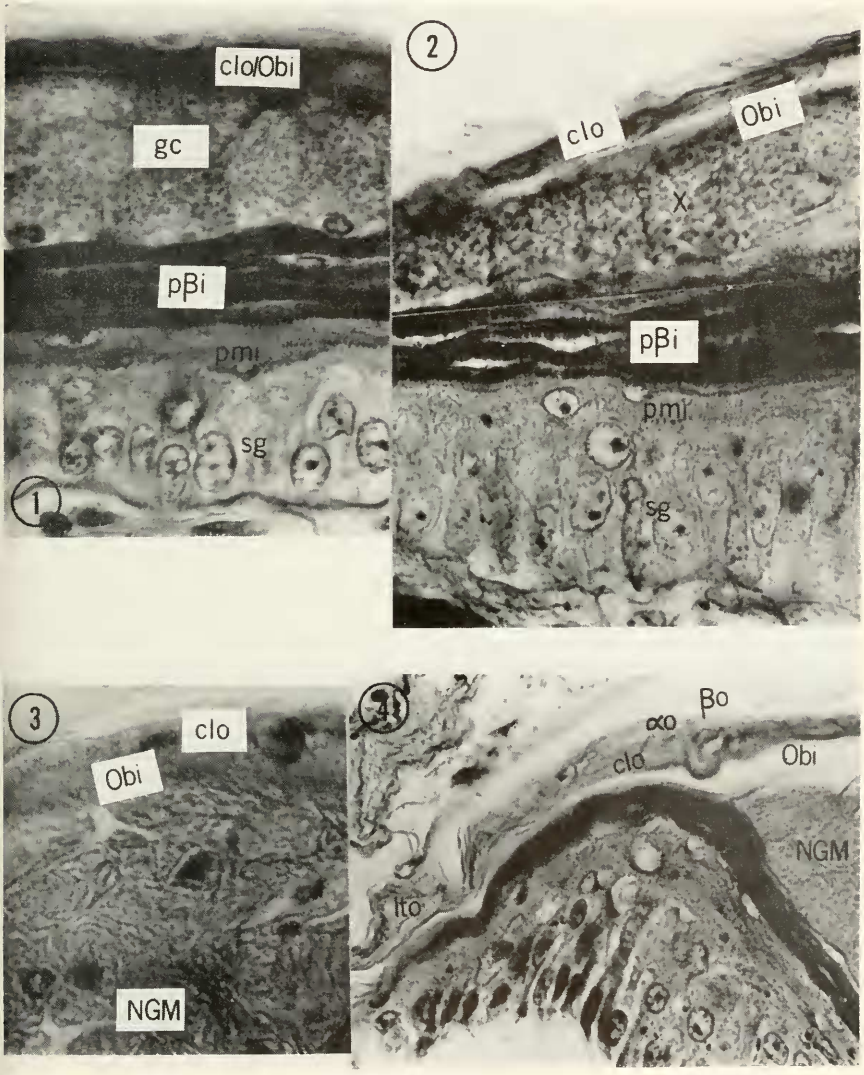
## PLATE 4

Figure 1. Micrograph of epidermis from an adjacent "semi-specialized scale" showing a modification of a "Stage Four-Five" (Maderson, 1966b) with the addition of two layers of granular cells (*gc*) between the *Oberhautchen* (*Obi*) and the rest of the presumptive $\beta$-layer ($p\beta i$). Aniline blue-orange G stain.

Figure 2. Similar region of a specialized scale, as seen in sagittal section, to that shown in Pl. 3, fig. 3. This specimen shows a slightly more advanced stage of histogenesis of the new $\beta$-layer. It is readily comparable to the condition shown in figure 1 above. Note that it is possible to see where the spinules of the new *Oberhautchen* have separated from the keratinized clear layer of the outer epidermal generation. Aniline blue-orange G stain.

Figure 3. Similar region to that indicated by rectangle marked *Z* in Pl. 2, fig. 4. Hematoxylin and eosin stain.

Figure 4. Similar region to that shown in fig. 2 in an early "Condition Four." Note the chromophobic appearance of the stratum germinativum in this figure and figs. 1 and 2; this feature is characteristic of the period before, during, and after the new mesos layer is laid down. Hematoxylin and eosin stain..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4
Bo
αo
clo
Obi
NGM
lto

PLATE 4

## PLATE 5

Figure 1. Extreme lateral margin of specialized scale from abdominal region, seen in transverse section, showing an immediate pre-slough condition (late "Condition Four"). The $\beta$-layer of the outer epidermal generation is absent from the photograph. The new *Oberhautchen* separates from the clear layer to facilitate sloughing, to the left of the picture. In this region there is a lacunar tissue (*lto*) above the clear layer. To the right of the picture, there is no development of the lacunar tissue, clear layer, or *Oberhautchen* so that the $\alpha$-layer of the outer epidermal generation (*ao*) separates directly from the new inner epidermal generation. This material will be exposed when sloughing occurs. The mesos layer (*mo*) of the inner epidermal generation has now keratinized and resembles the similar component of the outer generation. The stratum germinativum cells are regaining their chromophilic properties as the first presumptive $\alpha$-cells laid down ($p \propto i$). Hematoxylin and eosin stain.

Figure 2. Plan view of a sagittal section through the scale on which the pre-anal pore opens. The rectangles marked *a*, *b*, *c*, *d* are shown in figs. 3 and 4 and Pl. 6, figs. 1 and 2, respectively. Note that the scales anterior and posterior to the scale on which the pore opens show a late "Condition Three" of the $\beta$-glands, but the scale itself is unspecialized (see figs. 3 and 4). *H* indicates direction toward head, and *T* indicates direction towards tail. Hematoxylin and eosin stain.

Figure 3. Region shown in rectangle *a* in fig. 2. This shows the epidermis in a typical Stage Four condition (Maderson, 1966b). Note the sharp discontinuity of the development of the complex epidermal generation in the mouth of the pore as shown by the arrow. The fine vertical striations indicating the old (*Obo*) and new (*Obi*) *Oberhautchen* spinules can just be seen. Several fragments of the pre-anal pore material can be seen lying in displaced positions. The lumen of the pore in this and fig. 4 (below) is indicated by *L*.

Figure 4. Region shown in rectangle *b* in fig. 2. The epidermis here shows the modified Stage Four condition (Maderson, 1966b) with a greatly reduced $\beta$-layer which characterizes the inner scale surface. Note the much-enlarged clear layer cells and the enclosed granules which may be keratohyalin (see discussion, Maderson, 1966b). Again the sharp discontinuity of the epidermal generation in the pore mouth can be seen (arrow).

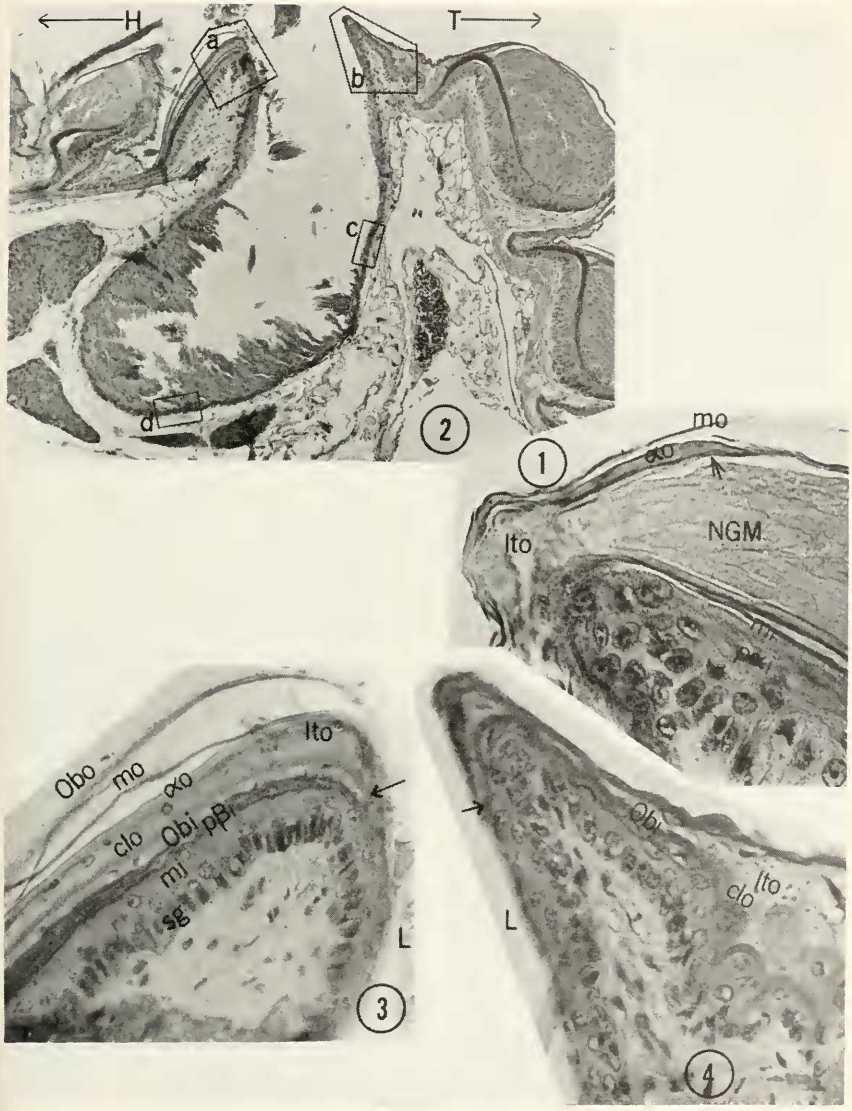
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PLATE 5

## PLATE 6

Figure 1. Region indicated by rectangle *c* in Pl. 5, fig. 2. *L* indicates the lumen of the pore and *D* the dermis. Note the complete absence of any indication of "epidermal generation" structure.

Figure 2. Region indicated by rectangle *d* in Pl. 5, fig. 2. This shows the cellular elements comprising the pre-anal pore secretion which are almost fully mature and keratinized. Two pycnotic nuclei are indicated by arrows.

Figure 3. Cells in the anterior region of the forward-running pre-anal pore where the cells are immature. Note the extremely conspicuous intracellular fibrils. Aniline blue-orange G stain.

Figure 4. Cells in the extreme anterior portion of the pre-anal pore; this region is apparently the "germinal region," the site of origin of all the pore secretion. As the cells arise from the germinal layer (*sg*) they show three conspicuous features. The nucleus lies towards the germinal layer. There is a central region where fibrils are just visible (*f*) which stain intensely with orange G. There is a distal region which is occupied by a large droplet (*Dr*) which stains with aniline blue. As the cells move away from the anterior part of the gland, the droplet disintegrates, giving the picture seen in fig. 3. Aniline blue-orange G stain.

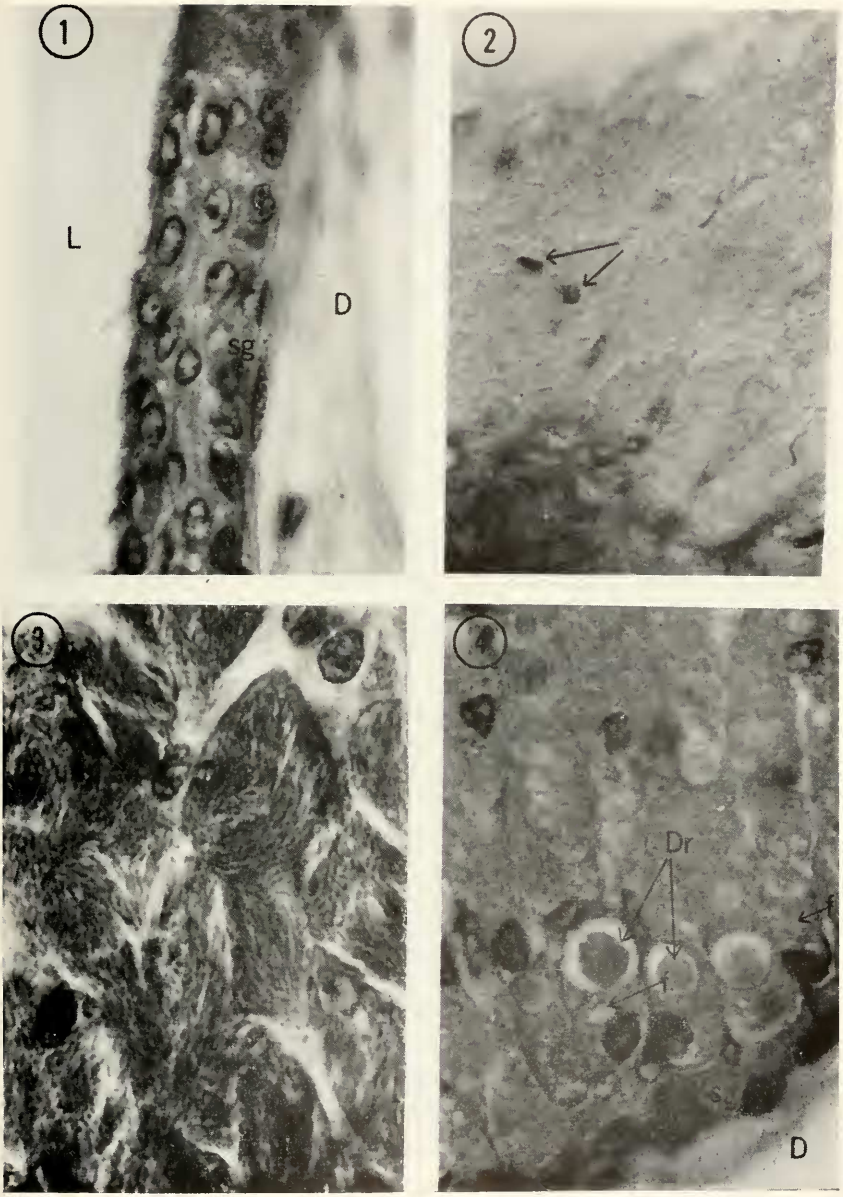


PLATE 6